EVALUATION OF MOUTHWASHES BASED ON PHYSICAL PARAMETERS USING ACONTACT ANGLE GONIOMETER- AN IN VITRO STUDY

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ABSTRACT

AIM

To evaluate the surface tension and contact angle to determine better wettability of three mouthwashes: chlorhexidine, povidone iodine and an oxygen releasing mouthwash mouthwash using a contact angle goniometer.

MATERIALS AND METHODS

For this in vitro study, 30 anterior (maxillary and mandibular central and lateral) teeth that were extracted from patients to be discarded were preserved and used. From these, 30 labial enamel plates were prepared, and divided into three groups (n=10 for each group), a drop of the mouthwash selected, i.e. povidone iodine, chlorhexidine and an oxygen releasing mouthwash was added onto the labial surface and the contact angle and surface tension of each mouthwash, was computed by the contact angle goniometer. The Right and Left Angle (RA and LA), Right and Left Contact Point (RCP and LCP) and Surface Tension were compared using the ANOVA test used to assess intergroup and intragroup statistical significance respectively; ($p<0.05$ was considered statistically significant).

RESULTS

A statistically significant difference was noticed in the Right Angle (RA) of chlorhexidine mouthwash. The Left Angle (LA), Left Contact Point (LCP) and Right Contact Point (RCP) values were statistically insignificant ($p>0.05$) while the lowest surface tension was demonstrated by povidone iodine.

CONCLUSION

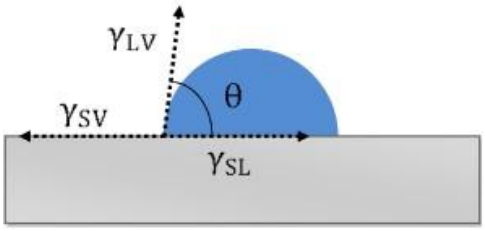
Povidone iodine demonstrated the lowest surface tension compared to Chlorhexidine and the oxygen releasing mouthwash suggesting increased wettability on a tooth surface followed by chlorhexidine and oxygen releasing mouthwash respectively.

KEYWORDS: Contact Angle, Wettability, Surface Tension, Mouthwash, Chlorhexidine, Povidone iodine

INTRODUCTION

Various liquids flow in different ways across different surfaces. The extent to which a fluid spreads onto a particular surface is important regarding disinfection especially in the field of dentistry as the oral cavity harbors multiple soft and hard tissue landmarks that are difficult to access. Disinfection, whether done as daily oral hygiene practices or most crucially, before surgical procedures requires the mouthwash used to reach inaccessible areas a property termed as wettability.[1] Wetting is the extent to which a liquid may spread over a solid surface, and it is measured by measuring the contact angle (CA) of the liquid to the solid surface. This measurement also helps determine hydrophobic and hydrophilic materials.[2] A strength balance between adhesive and cohesive forces determines the degree of wetting. The contact angle can help calculate surface free energy based on the following formula (figure 1).[3]

FIG 1: Schematic diagram of a contact angle of a liquid drop placed on a solid surface.



(Image courtesy: https://www.ossila.com/en-in/products/contact-angle-goniometer)

Surface tension is an attractive force which is imposed onto the surface molecules of a liquid by the molecules underneath the liquid that tends to attract surface molecules into the body of the liquid, causing it to adopt the form with the least surface area. A water droplet will acquire a perfectly spherical form in the absence of gravity and the impact of a solid in contact with the liquid; most surfactants modify the surface characteristics of the liquid but may not affect the composition of the liquid at all.[4] The contact angle is determined by a balanced adhesive force (the liquid's propensity to remain in contact with a solid) and a cohesive force within the liquid. [5],[6] An increase in the adhesive force between the liquid and the solid, or a decrease in the cohesion force in the liquid (surface pressure), resulting in a larger weight resistance and a smaller point of contact. It is vital to note that the adhesive force can change as a consequence of the fluid or fluid changes, but the cohesive force can only change as a result of a change in the fluid's surface strength or cohesive strength.[5]

A contact angle goniometer is an excellent indirect measure of surface wetness. When a drop of liquid begins to spread across a surface, the contact angle increases to 90 °. Wetting is complete when this drop of liquid is flat, or when the contact angle of the liquid onto the solid surface is zero degrees. A Contact angle goniometer may be used to produce optical tensiometer measurements by dangling a drop of the liquid chosen from the tip of a pointed needle and

comparing the sessile liquid drop models to the picture obtained by the instrument. A tilting stage, a light source that is monochromatic in nature, a high resolution camera, and the contact angle analysis software are the main components of the device.

The oral cavity harbors an array of micro organisms; aerobic and anaerobic through organic film layers called acquired pellicles which form on the dental hard tissues and prosthesis minutes after exposure to food or even after cleaning due to the attraction of these surfaces to salivary glycoproteins and bacterial products.[7] This dental pellicle matures to form what we know as plaque.[8] Some of the characteristic features of the pellicle and plaque seem to be related to the surface of the underlying solid surfaces and restorations by a certain adhesiveness that has been recorded using intraoral contact angle measurements and critical surface tensions.[9] It was reported that the adhesive properties of various restorative artificial surfaces with diverse surface chemistries were modified after two hours in the oral environment and produced in vivo critical surface tensions that are usually recorded for enamel and was attributed by the formation of this dental pellicle.[10] Intraoral disinfectants aim to reduce this plaque formation with antimicrobial effect.

Mouthwashes not only require low surface tension but maximum wettability and contact angle. Thus it enhances surface weight for maximum strength and destruction of bacterial cell walls.[11] Chlorhexidine is considered a gold standard based on the property of substantivity. It is also found to be bacteriostatic as well as bactericidal depending on its concentration. Povidone iodine is a product of the water-soluble polymer polyvinylpyrrolidone and iodine.

Povidone iodine antibacterial effect occurs when iodine dissociates from the combination. Iodine penetrates into the microbial cell membrane in its free state and then interacts with the proteins, nucleotides, and fatty acids in the cell wall. This chemical reaction eventually leads to cell destruction. Povidone-iodine has beneficial broad antibacterial action against gram-positive as well as negative bacteria, and various other microorganisms like fungi, tubercle bacilli, protozoa, viruses, bacterial spores etc.

Oxygen releasing mouthwashes work on the principle of controlled supply at the treatment site of active oxygen i.e. hydrogen peroxide. When sodium perborate comes into contact with water, a chemical process is created for hydrolysis, with hydrogen peroxide and boric acid as the finishing material and is widely used in patients undergoing implant therapy. Our team has extensive knowledge and research experience that has translated into high quality publications. [12–21]

Current literature exists explaining the antimicrobial effects of the commonly available mouthwashes and do not take into consideration the physical properties of these mouthwashes as liquid agents and their ability to coat the tooth surfaces, hence the aim of this in vitro study was to evaluate the surface tension and contact angle to determine better wettability among three mouthwashes: chlorhexidine, povidone iodine and blue®m mouthwash using a contact angle goniometer.

Materials and Methods:

1. Study setting and design:

This in-vitro study was conducted in the Department of Periodontology, over a period of three months, from October to December 2022. Two researchers (S.M. and I.P.) designed the study with the aid of the institution's material research laboratory personnel. The study design was approved in September 2022 by the institute scientific research board. As no human participants were involved in the study, no human ethical clearance was indicated. Inclusion criteria was as follows: extracted human anterior teeth obtained from healthy patients with severe periodontal disease that were indicated for extraction. Exclusion criteria: posterior teeth due to irregular surfaces which would give an inconsistent reading, teeth belonging to other species, carious teeth, teeth with enamel and dentin deformities, discolored and eroded teeth.

2. Preparing labial enamel plates:

30 extracted human maxillary and mandibular central and lateral incisors were obtained from the department of oral surgery. The obtained teeth were washed thoroughly and scaled. These teeth were then sectioned longitudinally to create thin enamel plates of approximately 10 mm in length. The thirty enamel plates were divided into 3 groups (n=10). The plates were then mounted on the stage of the contact angle goniometer (Ossila, Netherlands).

3. Measurement of contact angle and surface tension using a contact angle goniometer:

A drop of mouthwash; Chlorhexidine (Hexidine 0.2%), Povidone iodine (Xtracare 2%) and an oxygen releasing mouthwash (BlueM, Europe) was added onto the mounted labial enamel plates and using the pre-calibrated glass syringe that dispenses an exact droplet onto the enamel plate surface. A camera of 1920 X 1080 resolution takes a zoomed photograph of the mouthwash droplet on the tooth section and the software is run, the contact angle for each droplet is measured from the base of the enamel plate section and the line angle at which the droplet makes contact with the surface. The values for each were recorded. This is repeated with each labial enamel plate section and mouthwash.

4. Statistical analysis:

The sample size and calculations were carried out from a previous study. All data was taken from the study was assessed in the form of mean ± standard deviation statistical significance was assessed using IBM SPSS version 23 independent t test and ANOVA were used to assess intergroup and intragroup statistical significance respectively; ($p < 0.05$ was considered statistically significant)

RESULTS

A sessile drop on a smooth, flat and on a plane solid surface (ideal surface) demonstrates axial symmetry adopting the shape of a sphere. Sessile mouthwash drops were studied over extracted human central incisors to study the contact angle and surface tension of each from the right and left contact point for the mouthwashes (chlorhexidine, povidone iodine and oxygen releasing mouthwash)(figure 2-6). It was observed that povidone iodine had the lowest RCP and LCP compared to chlorhexidine and povidone iodine.

It was also observed that chlorhexidine and blue mouthwashes had similar RCP although the LCP of chlorhexidine was slightly higher than the oxygen releasing mouthwash.

Surface tension values of each mouthwash was calculated by the contact angle goniometer from which it was observed that povidone iodine had the least surface tension (Table 1) correlating from the contact point measurement of povidone iodine that the least contact point has the least surface tension and therefore increased wettability on a surface.

Figure 2: pendant drop image of povidone iodine taken by the software showing the surface tension value of 625.6mN/m

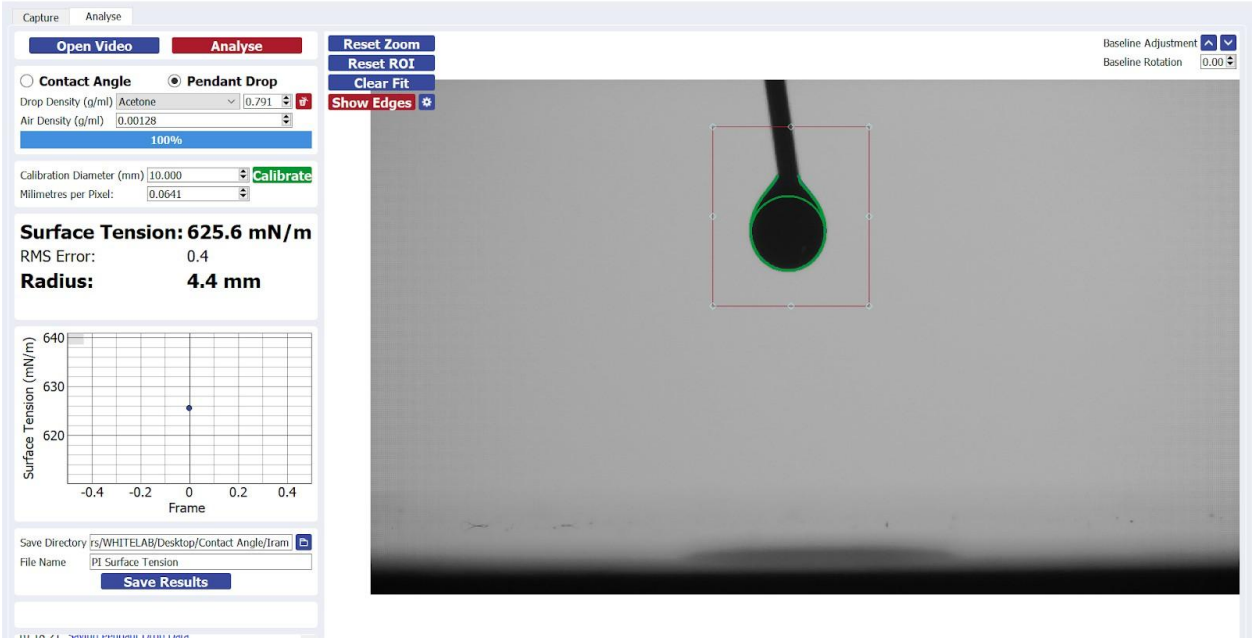


Figure 3: pendant drop image of chlorhexidine taken by the software showing the surface tension value of 849.0mN/m

Figure 4: contact angle measurements of a sessile drop of chlorhexidine shows the left and right contact angles

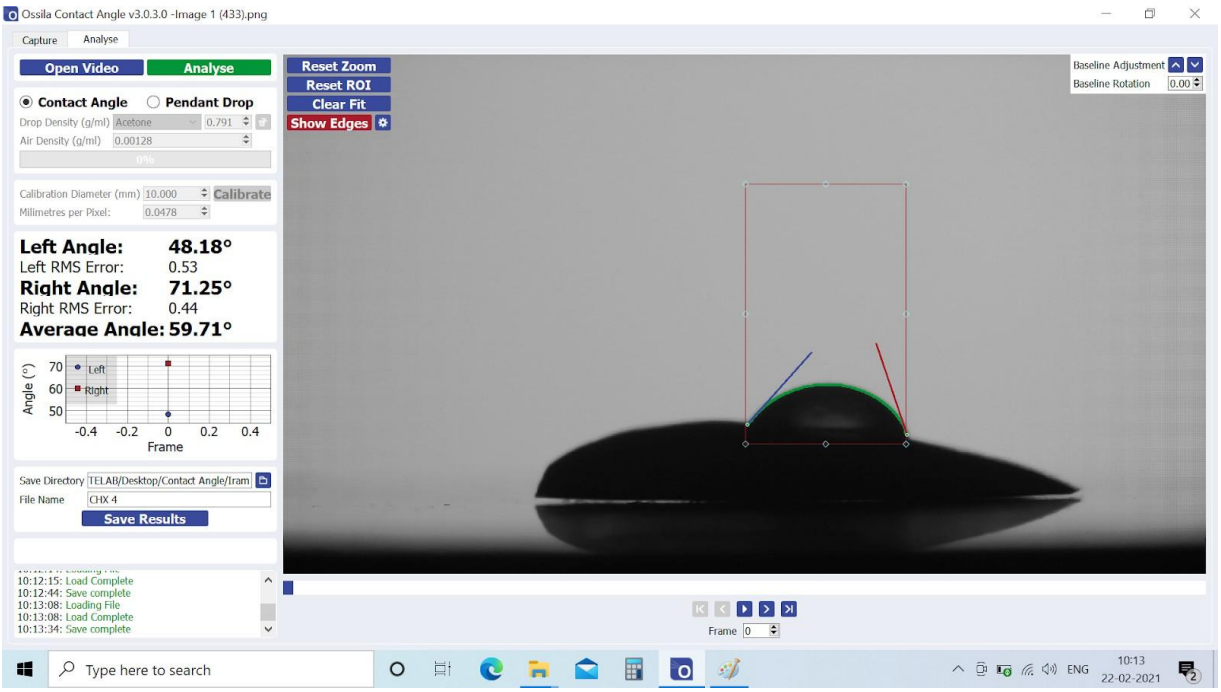


Figure 5: contact angle measurements of a sessile drop of Blue m mouthwash shows the left and right contact angles

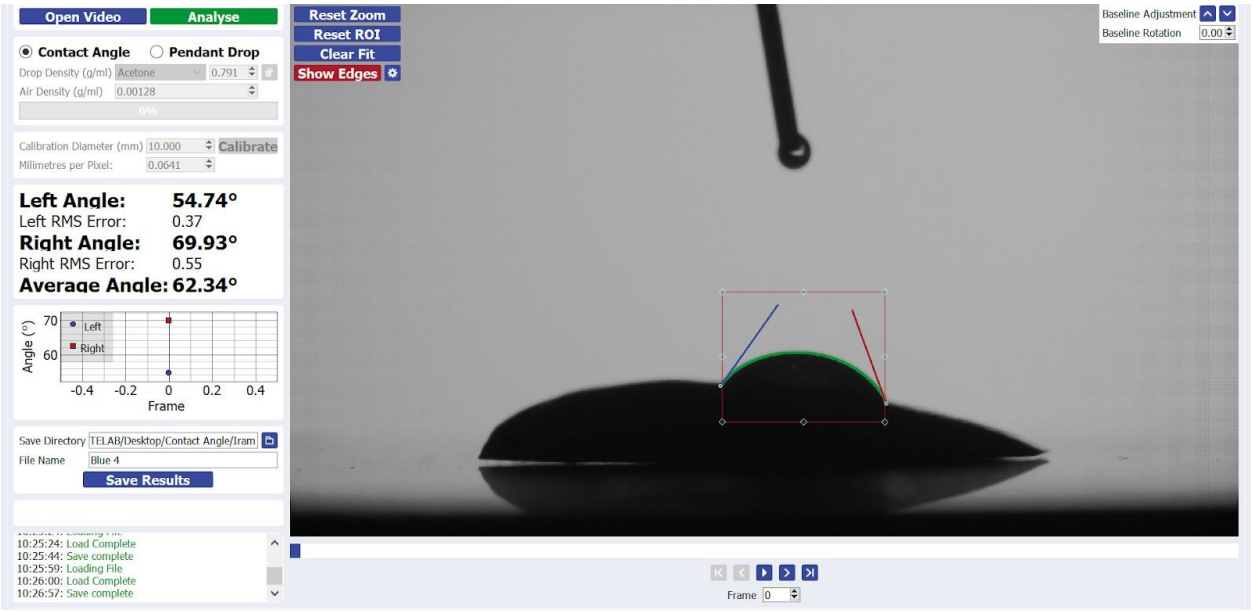
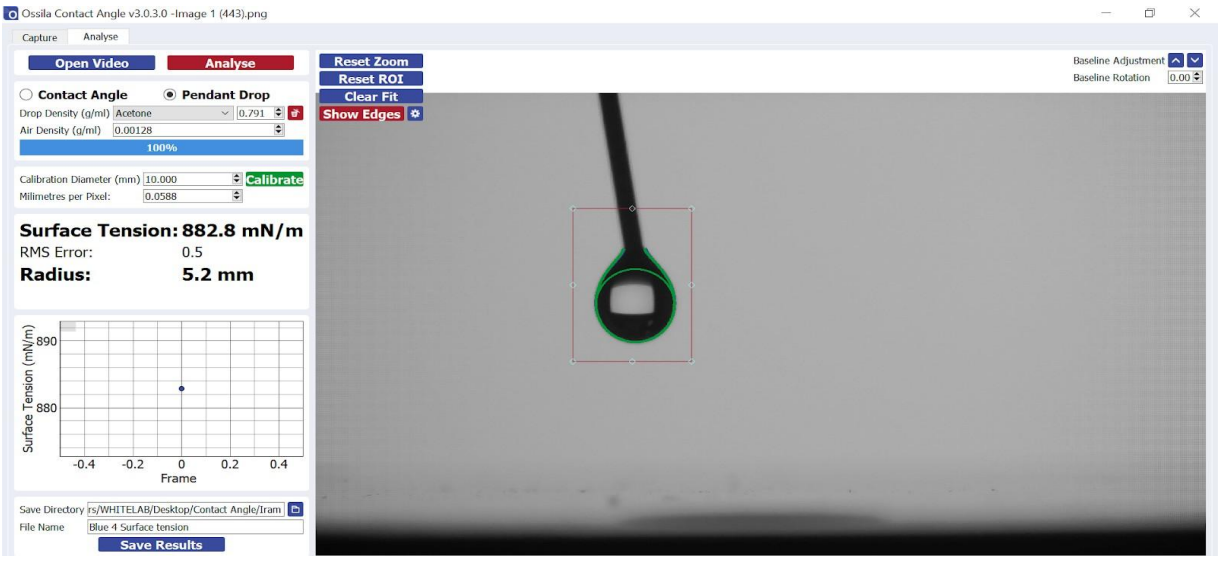
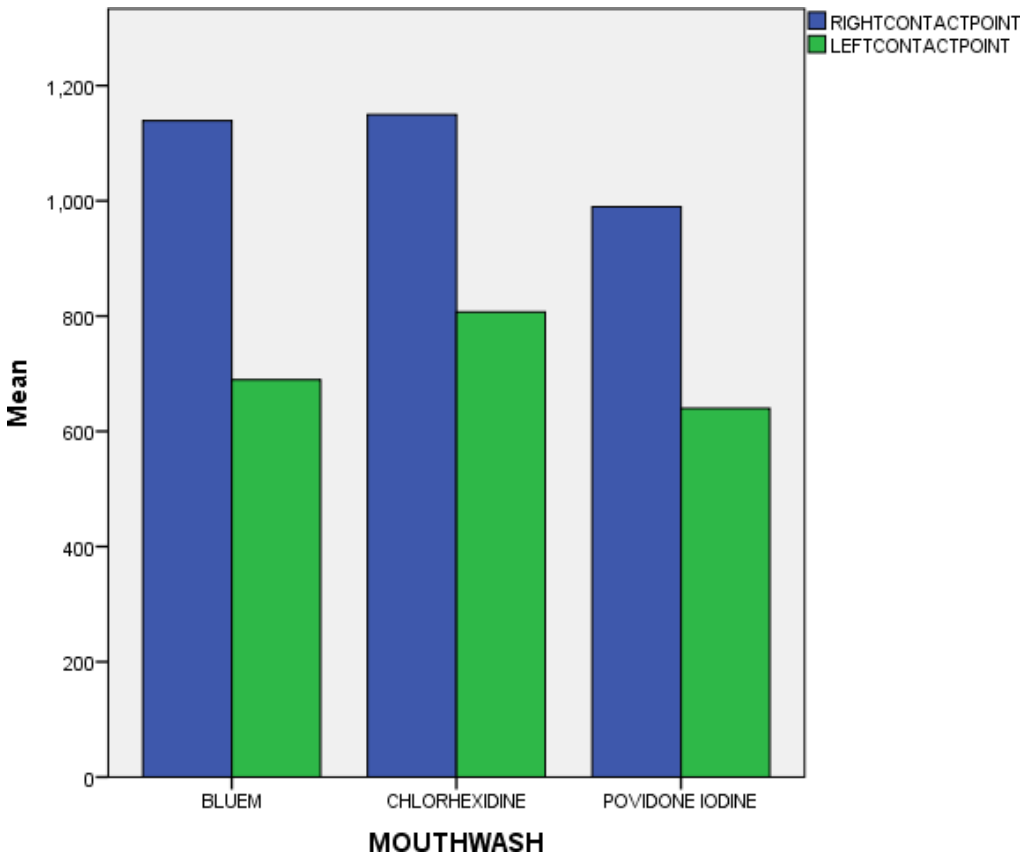


Figure 6: pendant drop image of the oxygen releasing mouthwash taken by the software showing the surface tension value of 882.8mN/m



Tables:

Figure 7: the following bar graph represents the right and left contact point for the mouthwashes (chlorhexidine, povidone iodine and blue®m mouthwash)



From the above bar graph, an increase in left contact point is seen with respect to chlorhexidine. Povidone iodine comparatively, shows the lowest right and left contact point.

Table 1: surface tension values calculated by the contact angle goniometer of the selected mouthwashes.

Mouthwash	Surface Tension (N/m)
Blue®m	882.8337901
Chlorhexidine	849.0035132
Povidone Iodine	625.5598896

Table 1 depicts the surface tension in newton/ meter for each of the selected mouthwashes; the lowest surface tension is demonstrated by povidone iodine

Table 2: group statistics for outcome measures

Groups	Outcome measures	Mean ± std. deviation	Significance (p value)
Chlorhexidine VS povidone iodine and blue®m	Left angle (LA)	9.69±0.48	NS
Chlorhexidine VS povidone iodine and blue®m	Right angle (RA)	303.092±0.750	*
Chlorhexidine VS povidone iodine and blue®m	Left contact point (LCP)	294.87±0.542	NS
Chlorhexidine VS povidone iodine and blue®m	Right contact point (RCP)	320.44±1.078	NS

Table 2 demonstrates the significance ($p<0.05$) of left and right angle; left and right contact point. A significant difference was noticed in the right angle of chlorhexidine mouthwash. NS- statistically insignificant ($p>0.05$) *Statistically significant ($p<0.05$)

DISCUSSION

The use of a mouthwash aims to reduce the microbial load of the oral cavity. A good mouthwash should have properties of wettability, antimicrobial activity, should sustain over longer periods of time and have physical properties such as low surface tension for maximum wettability not only over hard and soft tissues but restorations, prosthesis and interdental spaces. Studies have reported the influence of wettability, surface roughness on bacterial cell adhesion and subsequent cell wall destruction. These studies have reported that the adhesion of bacteria takes place on hydrophilic rather than hydrophobic surfaces thereby increasing the chances of biofilm development. [22-23,24]

One study reported that bacterial adhesion and colonization decreased as the surface became increasingly hydrophobic in nature on silicone samples with contact angles ranging from 110-141 degrees. Although it is critical to note that materials with increased surface roughness had a notable increase in bacterial colonization.[23,24-25]

From the above study done, it is an important observation that surface roughness of teeth in the oral environment may not be the same throughout. As age advances and wear and tear begins, changes in surface roughness could affect the surface tension of these mouthwashes. In one study, the transfer of bacteria from contact lenses to surfaces was studied with various surface wettability and roughness; and contact angles ranging from 30- 107 degrees.[26] They found that materials that exhibited the most roughness and highest contact angle harbored the

least amount of bacteria. These results are contradictory to a study where variable wettability and contact angle of various contact lenses to the bacterial adhesion of gram positive and gram negative bacteria was investigated. The contact angles ranged from 57-106 degrees and the wettability was measured using sessile drop technique. The results indicated that both strains adhered on to the more hydrophilic than the hydrophobic material.[25] These results from the above study are so conflicting that they indicate that there are various other factors than just variables of contact angle, surface tension and roughness that promote the bacterial adherence onto a surface like the intraoral soft and hard tissues.

A study where 98 hospitalized infants were scrubbed with chlorhexidine and povidone iodine, Skin cultures were collected before and after disinfection with the solutions. There was no significant difference with respect to microbial colony count before disinfection but a significant reduction in colony count after disinfection with povidone iodine when compared to chlorhexidine.[27] Contradicting results were seen in a study where 849 adults undergoing surgery were prepped with a chlorhexidine based surgical scrub or one with a povidone–iodine based. Outcome measures were any SSI (Surgical Site Infection) at any point of time within 30 days post surgery. It was observed that the chlorhexidine–alcohol scrub group demonstrated an overall lower rate of SSI than the povidone–iodine group. [28]

Another study established the efficiency of using a chlorhexidine antiseptic protocol versus using a povidone-iodine in reducing Surgical Site Infections for mothers undergoing cesarean deliveries. They also reported that the Chlorhexidine based scrub was significantly more effective than the povidone iodine scrub against superficial and deep incisional infections. [29] From the results of our study we observed that povidone iodine had the lowest surface tension and therefore had the maximum wettability. Although we cannot dismiss the chemical properties that chlorhexidine, being a gold standard mouthwash has, microbial analysis comparing various mouthwashes would help determine the correlation between physical and biological properties.

The results of our study demonstrate that povidone iodine exhibited the lowest surface tension; hence the lowest surface energy thereby increased wettability on a tooth surface. Chlorhexidine, a gold standard mouthwash, showed a higher surface tension than povidone iodine followed by oxygen releasing mouthwash. Static (sessile drop) contact angles were measured in this study. The droplet is taken on the surface (labial enamel plate) and the three phase boundary is static (solid liquid and gas). However a range of contact angles exist for a particular surface based on confounding factors like surface roughness, chemical structure and topography. To accurately attempt to gauge the range of contact angles for a particular surface, dynamic advancing and receding contact angles are measured; a.k.a. Contact angle hysteresis. Hysteresis occurs when the surface lacks homogeneity (like the surfaces of teeth and oral structures), presence of plaque, impurities in the solution or alteration of it on the solid surface.

Static contact angles measure the wettability of a surface. We observed that there was a significant difference in right angles with respect to chlorhexidine. The difference in contact angles at the right and left point could indicate advancing and receding contact differences between the three mouthwashes.Advancing and receding contact angles can have a difference as high as 50 degrees. This is important to note because these small sliding angles/ roll of angles have self cleansing applications.

1. Low surface tension; povidone iodine demonstrated the lowest surface tension amongst the three presurgical mouthwashes followed by chlorhexidine and the oxygen releasing mouthwash. One essential use of contact angle measurement is determining the typical surface free energy of a solid-surface (SFE). The surface free energy of a solid is identical to the surface tension of a liquid, with the same units (mN/m or dynes/cm). Although the contact angle provides a measurable indicator of a surface's wetting qualities, it is always dependent on the probe liquid utilized. Several contact angles and probe liquids are necessary to measure a surface's SFE, and this data may then be fit to existing theories to get surface free energy values.
2. Antimicrobial properties: the criteria above include physical properties; a mouthwash should have a strong antimicrobial agent. The mouthwashes used in our study are various commonly used agents before surgical procedures. They have to be fast acting, bactericidal and should not leave any residue.

From the above study it was demonstrated that povidone iodine having the lowest surface tension and therefore highest wettability was the mouthwash of choice for administration before intraoral surgical therapy according to its physical properties of fluid behavior.

Within the limitations of this study, Future scope could include correlation of antimicrobial parameters. This would indicate which mouthwash would; considering its physical properties from the study done above, also be effective in antimicrobial activity.

CONCLUSION

Povidone iodine demonstrated the lowest surface tension compared to Chlorhexidine and blue®m mouthwashes suggesting increased wettability on a tooth surface followed by chlorhexidine mouthwash, oxygen releasing mouthwash.

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